

THE PITUITARY REGULATION OF MELANOPHORES IN THE RATTLESNAKE

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INTRODUCTION

Only recently has the pituitary been linked with certain color changes in reptiles (Noble and Bradley, 1933; Kleinholz, 1935, 1938*a, b*; Parker, 1938). All of these observations are concerned with the lizard metachrosis and indicate that the pars intermedia of the pituitary gland plays an important rôle in the regulation of the melanophores. The present study reveals that a similar concept must be extended to the snake, since in this animal the dispersed phase of the melanophores is likewise dependent upon the pituitary secretion. Furthermore, certain aspects of the melanophores' arrangement and activity of the snake offer an interesting contrast to those described for the chromatophores of the lizard.

MATERIAL AND METHODS

This study deals primarily with the observations of the effect of hypophysectomies and subsequent replacement injections on the activity of the skin melanophores of the prairie rattlesnake, *Crotalus v. viridis* Raf. These specimens were collected in the eastern part of Wyoming. Similar experiments were later extended to several other species of snakes.

The operative removal of the snake pituitary is relatively simple, since the lower jaw can be retracted sufficiently to expose the entire roof of the oral cavity. Intraperitoneal injections of 10 per cent Nembutal served for anesthesia as recommended by Clark (1937). After the skin and muscles have been removed over the basi-sphenoid region the more exact site of the gland can usually be seen through the semi-transparent bone. The latter is chiseled away and then the whole gland may easily be removed. After this operation the bone capsule is replaced and the skin stitched. A strictly aseptic technique is not necessary.

The pars intermedia is very pale and may easily be distinguished from the highly vascular and pink pars anterior. Since the intermedia tissue in the rattlesnake is approximately one-third to one-fourth the size of the anterior lobe and rather loosely attached to the latter, it may be removed independently and completely.

The effect of intermedin (melanophore-dispersing hormone) on the operated animals was studied by means of intraperitoneal injections of an extract of the pars anterior of the chicken pituitary. This tissue contains an unusually high concentration of melanophore-dispersing hormone and was prepared and assayed according to the method of Kleinholz and Rahn (1939, 1940).

The effect of these various operations upon the ophidian melanophore could most easily be observed and studied in the living, anesthetized animal with a dissecting microscope, since the branches of these large chromatophores extend into the epidermal layer and are contrasted sharply with the homogeneous yellow background of the dermis. For permanent recordings of the various changes scales were clipped, fixed in alcohol and prepared as whole mounts. To supplement this series other scales were serially sectioned at 10 micra.

These operations yielded very striking results in the rattlesnake and led to similar experiments in other species of snakes. Although their gross color changes were not as striking, the melanophores responded in a similar manner to both hypophysectomies and injections. Altogether seven rattlesnakes were hypophysectomized and several of these animals were observed for four months after the operation. The other operated snakes included five garter snakes, *Thamnophis ordinoides*; one ribbon snake, *Thamnophis radix* (B. and G.); one bull snake, *Pituophis s. sayi* (Schlegel); and four Florida water snakes, *Natrix sipedon pictiventris* (Cope).

OBSERVATIONS

The background color of the prairie rattlesnake is a homogeneous light yellow color covered by various melanophores and pigmented epidermal cells whose groupings and concentrations form various light- and dark-brown scales. The latter are responsible for the typical color pattern of the rattlesnake (Fig. 2). Before describing the induced color changes, it seems desirable to consider first the histology of the rattlesnake skin, since the arrangement and activity of its melanophores do not correspond in all respects with those described for the lizards (von Geldern, 1921; Schmidt, 1917; et al.).

Epidermis

One of the outstanding features of this tissue is the occurrence of typical, branched melanophores and their processes which in the lizard seem to be confined almost entirely to the dermis. The epidermis is divided conveniently into two layers, the stratum germinativum and the stratum corneum.

The cells of the germinativum are cuboidal to squamous and arranged in two or three rows. Among these cells one finds the cell body and branches of a small melanophore which will be referred to as the epidermal melanophore (Figs. 1, 5), although the cell body may often lie just below the basement membrane. Whenever it appears in the epidermis, it is probably a migrant from below. One must recognize two varieties of this rather evenly branched epidermal melanophore. One is large (200 micra) and found chiefly on the dorsal side where it contributes to the color of the dark-brown scales (Fig. 5). The other epidermal melanophore is much smaller (130 micra) and confined chiefly to the lateral scales. This cell is more delicate in structure and contains relatively little melanin (Fig. 9).

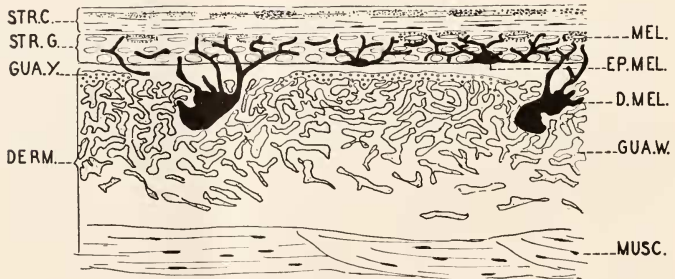


FIG. 1. Schematized section through a scale of the rattlesnake. The melanin deposits, *MEL.*, of the stratum corneum, *STR.C.*, are laid down first in the stratum germinativum, *STR.G.*, near the branches of the epidermal melanophore, *EP.MEL.*, and the dermal melanophore, *D.MEL.* The dermis, *DERM.*, contains a thick layer of white-reflecting guanophores, *GUA.W.*, covered by a thin layer of yellow-reflecting guanophores, *GUA.Y.* Muscle is denoted by *MUSC.*

The dermal melanophore (Figs. 1, 7) is discussed below. Yet, it must be briefly considered here as part of the epidermis, since its branches are found among the epidermal cells, while its cell body is always restricted to the dermis.

In the upper layer of the stratum germinativum the epidermal cells begin to keratinize and it is here that the first signs of intra and some inter-cellular melanin granules appear. Above this region is found the stratum corneum where the cells are completely keratinized and flattened. This region is destined to be cast at the next shedding. It has long been observed in older studies that this layer, the shedding skin, contains melanin granules which correspond in their horizontal distribution to the pigmented areas below. Whether or not these pigmented epidermal

cells (Figs. 1, 6) represent independent melanin producers is still questionable. At least they contribute greatly to the color intensity of the dark dorsal scales where they are especially prominent. However, they are not limited to this region alone, since they appear wherever melanophores occur and thus suggest a dependence on these cells.

Dermis

In this region are found the largest melanophores, the dermal melanophores (Figs. 1, 7) whose branches terminate among the epidermal cells. Their cell bodies lie imbedded among the dermal chromatophores responsible for the white-yellow background color of these animals. The latter pigment cells never extend into the epidermis. Their ramifying processes are filled with very small, alcohol-resistant crystals which reflect a white light and are doubly refractive under the polarizing microscope (*GUA.W.*, Fig. 1). Thus these cells may be regarded as guanophores according to the classification of Schmidt (1917). This white-reflecting guanophore stratum is in most places covered by a very thin layer of yellow-reflecting guanophores (*GUA.Y.*, Fig. 1) responsible for the almost homogeneous yellow background color exhibited by the scales. In a few places, this last-mentioned layer is absent (Fig. 1), such as the white-tipped dorsal scales and the regions directly above each dermal melanophore. In these places the dermis reflects only white color.

Effect of Hypophysectomy

The total removal of the pituitary as well as the extirpation of the pars intermedia alone causes a complete pigment concentration in all melanophore types. This would indicate that the pars intermedia is the only pituitary tissue responsible for the normally dispersed state of these cells. Evidence of a successful removal of this gland may be observed one hour post-operatively. Both types of melanophores begin to concentrate their pigment and the long branches of the dermal chromatophores give the impression of disappearing below the epidermis leaving small white islands in the otherwise yellow-reflecting guanophore area. After 4 to 24 hours the concentration is complete. The dermal melanophore pigment is clumped in an irregular fashion (Fig. 8). Granules of the guanophores do not seem to be affected.

It must be pointed out, however, that the snake as a whole does not necessarily appear lighter after the operation. This paling awaits the shedding of the stratum corneum, since this layer contains an abundance of already deposited melanin which may obscure any changes in the melanophores themselves. As soon as shedding has occurred, usually

three to four weeks after the operation, a striking change is observed (Fig. 2). (For similar observations on the induction of shedding in the snake after removal of the pituitary, see Schaefer (1933).) This paling is permanent, since little, if any, pigment is deposited hereafter.

Injection of Intermedin Hormone

The intraperitoneal injections of intermedin into an hypophysectomized animal cause complete dispersion in all melanophores. At least one hour is necessary before the first effects can be seen, yet 12 hours or more are required for the completion of this process (Figs. 4, 9). Large doses (several thousand *Anolis* units) will maintain complete dispersion for several days. The dermal melanophore dispersion, however, seems to be maintained by a lower intermedin concentration, since it may remain in this phase for several days after the epidermal melanophores have already completely concentrated their pigment.

It is interesting to observe that an hypophysectomized rattlesnake which has attained maximal pigment dispersion resulting from an intermedin injection is notably lighter than a normal animal (Figs. 3, 4). This emphasizes again that much of the color intensity of this animal is due to the melanin deposits in the keratinized portion of the epidermis and not entirely to the state of melanin dispersion in the chromatophores.

PLATE I

EXPLANATION OF FIGURES

2. Two normal (dark) and two hypophysectomized (light) prairie rattlesnakes. The control animal (*x*) has just shed and appears lighter than the other control animal which is about ready to cast its skin.

3. An hypophysectomized rattlesnake before the injection of intermedin hormone (anesthetized animal).

4. Same animal as in Fig. 3 photographed 24 hours after the injection of intermedin hormone. Notice that in spite of maximal pigment dispersion it is still lighter than the normal control animal in Fig. 2. (For explanation see text.)

Figs. 5-9. Photomicrographs taken from whole mounts of various scales. $\times 140$.

5. The large variety of epidermal melanophore which occurs primarily in the dark dorsal scales; maximal pigment dispersion.

6. Melanin containing epidermal cells (*m.*) from a dark, dorsal scale surrounded by epidermal melanophores (*ep. m.*) whose pigment is concentrated.

7. Dermal melanophores from a lateral scale, notice the bush-like appearance, finer branches, and lack of definite pattern.

8. Lateral scale of an hypophysectomized animal showing pigment concentration in the small variety of epidermal melanophore (*ep. m.*) and in the dermal melanophore (*d. m.*).

9. Lateral scale from same animal as Fig. 8; 6 hours after intermedin injection. Notice partial dispersion of pigment in both types of melanophores.

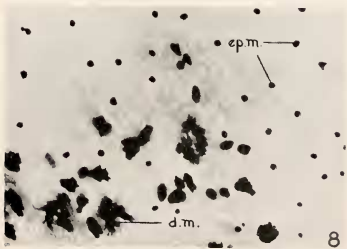
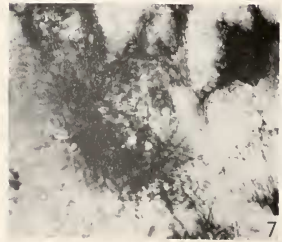
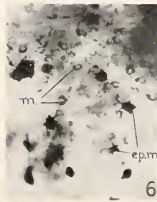
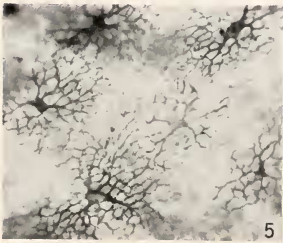
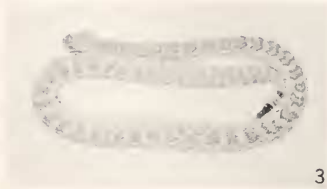
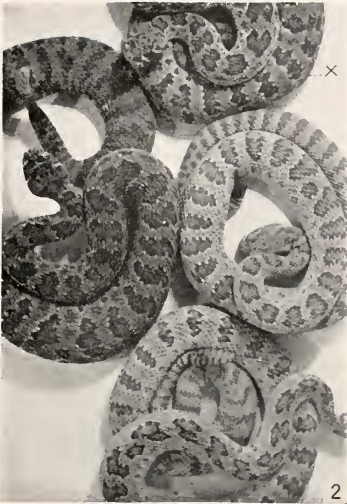


PLATE I

If intermedin injections were carried out for a considerable length of time in an hypophysectomized animal, one would expect to obtain the same dark color possessed by a normal animal due to the gradual deposition of epidermal melanin.

Other Snakes

A procedure similar to that outlined above was carried out with the other species of snakes mentioned. In all these forms suitable scales can be found which have small epidermal melanophores superimposed on a light dermis. These cells, however, account for very little, if any, color changes, yet will respond in the same way as the rattlesnake to hypophysectomy and intermedin injection. The color pattern of these snakes is accounted for primarily by a great concentration of various dermal chromatophores which have not been studied.

DISCUSSION

The primary concern of this study is to point out that the pituitary and more specifically the pars intermedia of the rattlesnake is responsible for the dispersed phase of both types of melanophores. Noble and Bradley (1933) were first to associate the paling response of the lizard, *Hemidactylus*, with the removal of the pituitary. Similar observations were later made in much greater detail on *Anolis* by Kleinholz (1935, 1938*a, b*), and on the horned toad, *Phrynosoma*, by Parker (1938). Little seems to be known concerning the normal color changes in snakes. The experimentally induced metachrosis described above is relatively slow but definite, and this group of reptiles may now be included in the ever increasing number of vertebrates which have a pituitary regulation of the melanophores.

The occurrence of epidermal melanophores and dermal melanophores whose processes reach into the epithelial layer has long been recognized, but has received little attention (Kerbert, 1877; Krauss, 1906; Fuchs, 1914; Schmidt, 1917; Lange, 1931). They seem to be rare in the lizards (Schmidt, 1917) and are probably obscured in most snakes by the dark, dermal chromatophore layer. In the rattlesnake, however, these melanophores are especially striking, since their grouping and distribution seem to be responsible for the whole color pattern superimposed upon a uniform yellow, dermal guanophore layer. The melanophores are not crowded and are easily seen, since all their branches extend into the epidermal stratum. This species is consequently peculiarly adapted for this study. The other snakes studied are on the whole rather dark-colored forms and the activity of the epidermal melanophores can only

be recognized in a few light scales and even here they contribute little to the color pattern.

All authors who have studied the shed reptile skin, especially that of the snakes (Leydig, 1873; Werner, 1892; Lange, 1931) have observed the close coincidence between the melanin pattern of the shed skin and the pattern of the underlying layers. This can be followed especially well in the rattlesnake where the melanin deposits of the stratum corneum appear to coincide with the spread of each individual dermal and epidermal melanophore. Such a situation poses the still unanswered question of how this melanin is deposited. Is the melanin formed independently by the epidermal cells, as seems to be the case in various regions of the amphibian skin, or is it actually "injected" into the epidermal elements by the branches of the melanophores as Strong (1902) has described for the birds? In the snake the close association of all melanophore branches with pigmented epidermal cells suggests very strongly a mechanism of melanin deposition as in the bird feather. If this is actually the case, then one could expect complete cessation of all pigment deposits in an hypophysectomized animal, since the pigment is completely concentrated and would never reach the upper layers of the stratum germinativum where it seems to be normally laid down. Although all hypophysectomized animals remained permanently pale as long as four months after the operation, closer examination revealed slight melanin deposits in the epidermal cells. However, this does not necessarily invalidate this theory, since all these snakes received periodic intermedin injections and consequently might have had an opportunity to deposit pigment during these intervals of melanophore dispersion.

To what extent the color changes in the snakes can be compared with the relatively sudden changes described for the lizard group is difficult to state. In *Anolis* (von Geldern, 1921; Kleinholz, 1938a) the light (green) and the dark (brown) phases are due to the concentration and dispersion of pigment in the dermal melanophores. These melanophores never extend into the epidermis and achieve their effect by exposing or masking the green-reflecting chromatophores of the dermis. This metachrosis may be accomplished in a matter of minutes. In the rattlesnake the light and dark phases are accomplished experimentally in a similar manner, but the processes of the melanophores lie primarily in the epidermis, give rise themselves to definite patterns and are rather slow to react. As pointed out before, another factor must be considered in the light and dark phase of the snake. This is the heavy accumulation of melanin granules in the epidermal cells, which plays a minor or questionable rôle in *Anolis*. The melanin accumulation is especially noticeable in the darkening of snakes before they shed (Fig. 2), for at this

time the future stratum corneum has already formed under the old layer and undoubtedly contributes to the darkened condition as the new layer already carries considerable pigment. The amount of melanin deposits, however, seems to be in some way correlated with the melanophore activity, since hypophysectomized animals lay down very slight amounts of pigment, or no pigment at all, and remain permanently pale.

Exact studies concerning the effect of temperature and light on the chromatophore activity in snakes have not been found in the literature. The rôle of these two factors in the melanophore regulation of various lizards has recently received much attention by Kleinholz (1938*a, b*), Parker (1938), and Atsatt (1939), but whether or not snake melanophores will respond in a similar manner awaits further study.

SUMMARY

1. In the prairie rattlesnake, *Crotalus v. viridis* Raf., the background of the skin is a homogeneous yellow-white color reflected from the evenly distributed dermal guanophores. The dark pattern of this snake is formed by various distributions of melanophores and pigmented epidermal cells superimposed upon this background.

2. Two main types of melanophores are found in the skin. One is relatively small and resides primarily in the epidermis; the other is much larger, structurally different, and retains its cell body in the dermis, but sends its ramifying processes into the epidermal layer.

3. Both of these melanophores appear to be associated with the deposition of melanin granules in the keratinizing portion of the epidermis, since (*a*) the distribution of its melanin deposits coincides with the pattern of the underlying melanophores, and (*b*) the rate of pigment deposition is greatly reduced after hypophysectomy.

4. The removal of the pituitary or the pars intermedia alone causes a permanent paling due to the complete concentration of the melanophore pigment. This paling, however, is more evident after the shedding of the old keratinized epidermal layer carrying previously deposited melanin.

5. Intraperitoneal injections of intermedin from the chicken pituitary will produce complete melanin dispersion in the melanophores of an hypophysectomized animal.

6. Preliminary observations on four other species of snakes indicate a similar pituitary regulation of the epidermal melanophores.

LITERATURE CITED

- ATSATT, SARAH R., 1939. Color changes as controlled by temperature and light in the lizards of the desert region of Southern California. *Publ. Univ. Calif. Los Angeles, Biol. Sci.*, 1: 237-276.

- CLARK, H., 1937. Embryonic series in snakes. *Science*, **85**: 569-570.
- FUCHS, R. F., 1914. Der Farbenwechsel und ihre chromatische Hautfunktion der Tiere. *Winterstein: Handb. vergl. Physiol.*, **3**: 1189-1656.
- VON GELDERN, C. E., 1921. Color changes and structure of the skin of *Anolis carolinensis*. *Proc. Calif. Acad. Sci.*, **10**: 77-117.
- KERBERT, C. 1877. Ueber die Haut der Reptilien und anderer Wirbelthiere. *Arch. mikr. Anat.*, **13**: 205-262.
- KLEINHOLZ, L. H., 1935. The melanophore-dispersing principle in the hypophysis of *Fundulus heteroclitus*. *Biol. Bull.*, **69**: 379-390.
- , 1938a. Studies in reptilian colour changes. II. The pituitary and adrenal glands in the regulation of the melanophores of *Anolis carolinensis*. *Jour. Exper. Biol.*, **15**: 474-491.
- , 1938b. Studies in reptilian colour changes. III. Control of the light phase and behaviour of isolated skin. *Jour. Exper. Biol.*, **15**: 492-499.
- KLEINHOLZ, L. H., AND H. RAHN, 1939. The distribution of intermedin in the pars anterior of the chicken pituitary. *Proc. Nat. Acad. Sci.*, **25**: 145-147.
- , 1940. The distribution of intermedin: a new biological method of assay and results of tests under normal and experimental conditions. *Anat. Rec.*, **76**: 157-172.
- KRAUSS, F., 1906. Der Zusammenhang zwischen Epidermis und Cutis bei Sauriern und Krokodilen. *Arch. mikr. Anat.*, **67**: 319-363.
- LANGE, B., 1931. Integument der Sauropsiden. Bolk, Göppert, Kallius und Lubosch: *Handb. vergl. Anat. d. Wirbeltiere*, **1**: 375-448.
- LEYDIG, F., 1873. Ueber die äusseren Bedeckungen der Reptilien und Amphibien. *Arch. mikr. Anat.*, **9**: 753-794.
- NOBLE, G. K., AND H. T. BRADLEY, 1933. The relation of the thyroid and the hypophysis to the molting process in the lizard, *Hemidactylus brookii*. *Biol. Bull.*, **64**: 289-298.
- PARKER, G. H., 1938. The colour changes in lizards, particularly in *Phrynosoma*. *Jour. Exper. Biol.*, **15**: 48-73.
- SCHAEFER, W. H., 1933. Hypophysectomy and thyroidectomy of snakes. *Proc. Soc. Exper. Biol. Med.*, **30**: 1363-1365.
- SCHMIDT, W. J., 1917. Die Chromatophoren der Reptilienhaut. *Arch. mikr. Anat.*, **90**: 98-259.
- STRONG, R. M., 1902. The development of color in the definitive feather. *Bull. Mus. Comp. Zool. Harvard*, **40**: 147-184.
- WERNER, FR., 1892. Untersuchungen über die Zeichnung der Wirbelthiere. *Zool. Jahrb., Abt. Syst.*, **6**: 155-229.